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Factors influencing bacterial eggshell contamination in conventional cages, furnished cages and free-range systems for laying hens under commercial conditions

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Abstract 1. The aim was to assess eggshell contamination in various laying hen-housing systems and to identify factors influencing this contamination.
2. Fifty-eight laying hen farms in France were studied, including 21 flocks housed in conventional cages, 7 in furnished cages and 30 kept on-floor.
3. Sixty eggs per flock were analysed to obtain counts of the total mesophilic flora. Data on equipment and hen management were collected.
4. Mean bacterial count on eggshells tended to be higher in on-floor systems ($4{\cdot}82 \pm 0{\cdot}51$ log CFU/eggshell) than in cage systems ($4{\cdot}57 \pm 0{\cdot}58$ log CFU/eggshell, $P = 0{\cdot}09$).
5. Contamination increased with age of the hens, airborne dust concentration, manual packing of the eggs, and packing in plastic rather than in recycled-pulp egg-flats.
6. The effect of the housing system on eggshell contamination, previously described in experimental assays, was confirmed under production conditions.

INTRODUCTION

To improve animal welfare, European Directive 1999/74/EC requires the ban of conventional cages for housing laying hens from 2012 onwards. Alternatives such as furnished cages or alternative systems (aviary systems, perchery systems, free range, etc.) have been proposed. In France, 80% of laying hens are still kept in cages (Magdelaine, 2006), but conventional cages are gradually being replaced by furnished cages, which include a nest box, a pecking and scratching area, and 15 cm of perch per bird. The most frequent alternative system in France is the on-floor hen house where the building is divided into a slatted area with perches and nest boxes and a litter area, while the hens frequently have access to an open-air range. Although these alternative systems have been evaluated in terms of production performance and welfare (Abrahamsson and Tauson, 1998; Tauson, 1998; Wall *et al.*, 2002; Michel and Huonnic, 2003; Guesdon *et al.*, 2006), few results are available on the effect of this change in housing conditions on bacterial contamination of the eggshell of eggs produced in commercial conditions. Studies under experimental conditions suggest eggs laid in alternative systems and in furnished cages are more contaminated than in conventional cages (Harry, 1963; Protais *et al.*, 2003*a*; de Reu *et al.*, 2005*b*; Mallet *et al.*, 2006). In the case of healthy hens, the egg content is generally free from microorganisms when laid (Mayes and Takeballi, 1983), but the eggshell rapidly becomes contaminated after laying due to contact with an environment soiled by faeces and dust (Board *et al.*, 1964; Quarles *et al.*, 1970; Gentry and Quarles, 1972). Eggshell bacterial load could have an impact on shelf life and food safety as penetration of the shell by bacteria present on its

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surface may lead to actual contamination of the egg (Harry, 1963; de Buck *et al.*, 2004).

The objective of the present study was to assess eggshell contamination in commercial production from various laying hen-housing systems in France and to identify the factors in laying hen-rearing management and egg-handling practices influencing this contamination. The systems studied were those currently available in France and included conventional cages, furnished cages complying with the requirements of Directive 1999/74/EC, and three alternative systems: on-floor in a barn without an open-air range, free range and organic.

MATERIAL AND METHODS

Study design and flock sample

The study was based on a cross-sectional survey carried out on a sample of laying hen flocks stratified according to housing system. The aim was to study 30 farms with cage systems (both conventional and furnished cages) and 30 farms with on-floor systems (barn, free range and organic). Farms selected were among those affiliated with the main egg production companies in Brittany (Western France) which agreed to participate in the study. Brittany is the primary egg-producing region in France (about 36% of French production in 2003; French Ministry of Agriculture).

Data collection

Each farm was visited once by two technicians trained in data collection and egg sampling. Data on design equipment, laying hen management and egg-handling practices were collected using a questionnaire (Table 1), which was filled in by the investigator during an interview with the farmer. The questions were adapted to describe each housing system studied. The cage questionnaire consisted of 55 questions of which 81% were closed-ended. The on-floor questionnaire included 65 questions (77% closed-ended). During the farm visit, the air quality in the laying hens' living area was assessed by monitoring temperature, ammonia concentration (an ACURO 2000 pump with Draëger reagent; France) and dust concentration for a whole day. Aerial dust was sampled with an air sampler CAP 10 (ARELCO; France) containing a filter to select dust particles less than 5 µm in diameter. Data on equipment, laying hen management, egg handling and air quality were transferred to an ACCESS 2000 database for analysis.

Table 1. *Summary of items included in the questionnaire to identify factors influencing eggshell contamination in various laying hen-housing systems (58 flocks, France, 2006-2007)*

General items related to the farm (5)
Farm staff characteristics
Location
Animal productions on the farm
Egg production on the farm (type, number of poultry houses, all-in all-out practice)
Items related to the poultry house (cage, 15; on-floor, 25)
Size
Building characteristics
Feeding, watering, and manure-disposal systems
Description of batteries and cages (cage flocks)
Description of nests, perches and litter area (on-floor flocks)
General characteristics and management of the flock under study (18)
Cleaning and disinfection procedures before pullet loading
Feeding, watering and lighting management
Hygiene procedures (dead bird disposal, staff clothes footwear, wildlife control)
Genetic strain of pullets
Productivity
Egg-handling practices (24)
Description of gathering system
Sorting and packing practices
Storage conditions
Cleaning and disinfection of egg machinery
Items related to the farm visit and to samples (3)
Season
Hen age at sampling
Transport time of samples from farm to laboratory

The number of questions per subset is indicated in parentheses.

Egg sampling

To ensure that the results were statistically reliable, 60 sorted eggs (dirty and macro-cracked eggs were removed) were sampled on each farm and eggs laid out-of-nest in alternative systems were excluded (de Reu *et al.*, 2005*a*). The eggs were sampled from the day's production after sorting and packing. They were taken from the compartments of the pack with sterile gloves and placed in new filler-flats. The eggs were taken by car in ambient conditions to the laboratory LDA 35 (Rennes, France). Eggs were kept for a maximum of 48 h in ambient conditions before analysis.

Bacteriological analysis

The eggs were pooled in 20 batches of three. These three intact eggs were placed in a plastic bag containing 200 ml of buffered peptone water and the shells were gently rubbed for 2 min (Protais *et al.*, 2003*a*). This primary solution was used to prepare a 1/10 dilution in tryptone salt medium. A total of 100 µl of the 1/10 solution were seeded on PCA agar (BioRad, France) using a spiral platter (WASP 2, AES, France) and incubated at 30°C for 48 h.

Statistical analysis

The mesophilic aerobic bacterial count per egg was transformed into $\log_{10}$ CFU/egg for statistical analysis. The recovery of zero CFU (colony-forming unit) corresponded to <3·8 $\log_{10}$ CFU/eggshell. Because this value could not be directly used for statistical analysis, the value of 3·8 $\log_{10}$ CFU/eggshell was used as previously suggested by Knape *et al.* (2002). Eggshell contaminations were compared between housing systems by a Kruskal–Wallis non-parametric test. Factors influencing mean eggshell contamination were identified using linear models. The dependent variable expressing eggshell contamination was the average of the log-transformed counts of the 20 egg pools analysed per flock. The predictors were the variables obtained from the questionnaires and from the air-quality measures. For the categorical variables, the numbers of categories per variable were limited so that the frequency rates of the categories were greater than 5%. The effect of categorical variables was assessed using a one-way analysis of variance (ANOVA) (PROC GLM, SAS 9.1). For the quantitative explanatory variables, a simple linear regression model was used. The fit of the model was verified visually for each significant factor ($P < 0·05$), the equality of variance was checked by plotting the standardized residues against the predicted values and the normality of the residuals by a normal probability plot. Influential observations were detected after examining Cook's distances and difference in fit (DFITS > 0·5), but no observation was removed.

RESULTS

Twenty-eight flocks housed in cages and 30 flocks kept in on-floor systems were studied in Brittany from September 2006 to October 2007. The cage farms housed between 10 192 and 65 000 laying hens (mean = 38 572 ± 15 120) with a dynamic ventilation system in 26 farms and a static ventilation system in two farms. Buildings were equipped with battery cages and the dropping disposal systems consisted of dropping belts in 24 houses and dip pits in 4 farms. Hens on 7 farms were housed in cages furnished with a nest box and perches. On two farms there was a pecking and foraging area with feed as the pecking material. The number of hens per cage in the furnished cage systems varied from 10 to 60 hens compared with 4 to 17 hens in conventional cages. The eggs in all these farms were collected from roll-out cages by belt conveyors which took them to a lift conveyor or elevators. A second conveyor transported the eggs from the poultry house to the room where they were sorted and packed in recycled pulp or plastic filler-flats. In 7 farms the eggs were marked before packing. After packing the eggs were stocked in a warehouse before collection and transport to a grading centre.

Twelve of the 30 on-floor farms specialized in organic egg production and 17 in free-range production. The last flock was kept in a barn and was the only one kept on-floor with no access to an open-air range. On-floor houses contained between 2889 and 10 662 (mean = 5830 ± 1947) laying hens, with an average density of 9·7 ± 2·7 hens/m^2. The buildings were divided into a slatted area, covering a pit for manure collection, and a litter area. All houses had a static aeration system. Eggs were manually collected and packed in 10 of these farms: eggs were gathered on the roll-out from the nest boxes by the farmer and directly sorted and packed on a trolley. On the other farms, eggs were gathered from roll-out nest boxes on to a cross-belt conveyor and transported to a sorting table in a separate room where they were sorted and packed. The most frequent genetic strain of hens, in both cage and on-floor farms, was ISA Brown. The average age of the hens on the day of sampling was similar in cage flocks and on-floor flocks (48·5 ± 17·7 weeks in cage versus 44·4 ± 14·1 on-floor).

The Figure shows mean shell contamination by system of production. Mean eggshell contamination tended to be higher in on-floor flocks than in cage flocks ($P = 0·09$) (Table 2). The difference in contamination between eggs from on-floor flocks and eggs from cage flocks was significant when comparing on-floor systems with conventional cage system ($P = 0·03$), but not when comparing on-floor systems with furnished cage system ($P = 0·33$). Within each type of housing system there was no difference of shell contamination between free-range and organic flocks ($P = 0·49$), whereas in cage systems contamination was higher in furnished cages than in conventional cages ($P = 0·02$). The effect of housing system is confirmed by results of ANOVA (Table 3): the average bacterial loads on eggs produced in furnished cages and in on-floor systems were respectively higher by 0·69 log CFU ($_{IC95\%}$ [0·24–1·13]) and by 0·41 log CFU ($_{IC95\%}$ [0·12–0·70]) than those of eggs from conventional cages. In addition, bacterial load significantly increased with hen age, the volume of air available per hen in the poultry house and the aerial dust concentration in the living area. Three significant factors were related egg handling. In 10 farms equipped with an automatic egg-gathering system, the egg belts were moved progressively during the day and this measure was associated with greater shell contamination. Mean bacterial count was higher when eggs were packed manually rather than automatically and

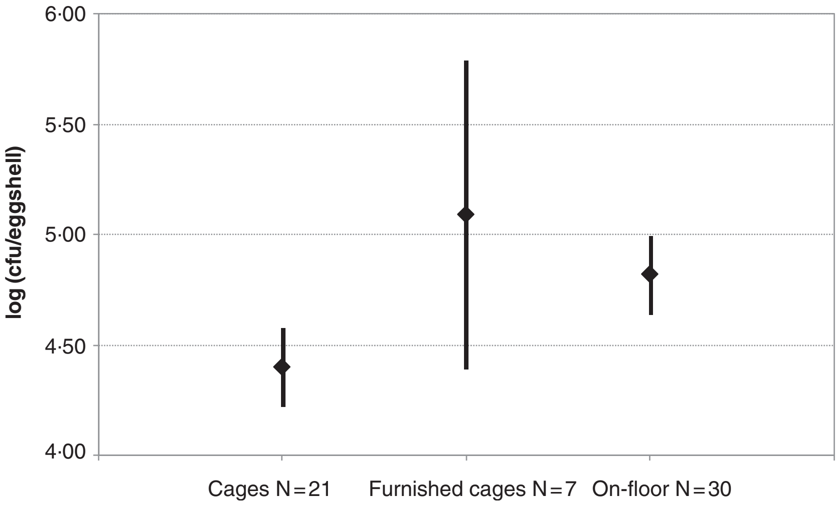

Figure. *Total count of aerobic flora on the eggshell in various laying hen-housing systems (58 flocks, France, 2006-2007). Vertical bars denote 95% confidence intervals*

Table 2. *Comparisons of mean counts of aerobic flora on the eggshell (95% confident intervals) between-housing systems (58 flocks, France, 2006-2007)*

Housing system	n	Mean count (log CFU/eggshell)		P
Cage	28	4·57	(4·35–4·80)	
Conventional cage	21	4·40[a]	(4·22–4·58)	
Furnished cage	7	5·09[b]	(4·38–5·79)	0·09
On-floor	30	4·82	(4·63–5·00)	
Free-range or barn	18	4·86[b]	(4·58–5·13)	
Organic	12	4·79[b]	(4·51–5·07)	

[a,b]Means within a column followed by different letters differ at $P < 0{\cdot}05$.

when they were packed in plastic rather than recycled pulp-flats.

DISCUSSION

The main objective was to assess shell contamination of eggs produced in various hen-housing systems under commercial conditions. Shell contamination in furnished cages and in alternative systems has previously been compared with that of eggs produced in conventional cages, but only under experimental conditions (Harry, 1963; Protais *et al.*, 2003*a*; de Reu *et al.*, 2005*b*; Mallet *et al.*, 2006) or in only a small number of farms (de Reu *et al.*, 2006). The mean bacterial loads measured in cage flocks and on-floor flocks in our survey were similar to those observed in experimental studies or in assays of eggshell decontamination in commercial premises (Lucore *et al.*, 1997; Knape *et al.*, 2002). It could be concluded from the larger sample of flocks that the shell contamination of eggs produced in commercial conditions and before transport to the grading centre would not exceed 5 log CFU/eggshell, except in furnished cages. A higher shell bacterial count on eggs from organic and free-range farms had been previously reported by de Reu *et al.* (2005*b*) in experimental premises and in field conditions (de Reu *et al.*, 2006). In the field study, as in the present survey, the difference in contamination between-housing systems was slight but significant, whereas in experimental conditions this difference could exceed 1 log CFU/eggshell.

The poorer bacteriological quality of eggs produced in alternative housing systems has frequently been related to floor laying. Floor eggs are more likely to be damaged or soiled and are therefore more contaminated (Protais *et al.*, 2003*a*; Sander *et al.*, 2003; de Reu *et al.*, 2006). In the present study, only eggs laid in nest boxes were analysed, so any differences observed between on-floor systems and the conventional cage system can be related to housing and environmental conditions rather than to floor laying. Interestingly, the aerial dust concentration appeared to influence greatly eggshell contamination in this study. Aerial dust monitoring had shown that the dust concentration was higher in on-floor hen houses than in conventional cage poultry houses. Takai *et al.* (1998), Ellen *et al.* (2000) and Guillam *et al.* (2007) also reported higher dust concentrations in perchery and aviary systems than in cage poultry houses. Because dust contains bacteria (Lyngtveit and Eduard, 1997; Radon *et al.*, 2002), the airborne bacterial concentration in on-floor premises is likely to be higher than in conventional cage hen houses (Protais *et al.*, 2003*b*; de Reu *et al.*, 2005*b*). This poor microbiological air quality in alternative housing systems may affect the bacterial concentration on the eggs (Quarles *et al.*, 1970).

Table 3. *Explanatory variables significantly linked to eggshell contamination in caged and on-floor systems (58 flocks, France, 2006-2007)*

Variables	*n*	Coefficient	SD[a]	CI[b]	*P*
Housing system					
On-floor	30	0·41	0·14	0·12–0·70	0·005
Furnished cage	7	0·69	0·22	0·24–1·13	0·003
Conventional cage	21	–		–	
Egg belts moving during the day					
Yes	10	0·37	0·19	–0·01–0·74	0·05
No	48	–		–	
Marking eggs system					
Yes	7	–0·43	0·22	–0·86–0·01	0·05
No	51	–		–	
Egg-packing system					
Automatic	27	–0·42	0·13	–0·69 to –0·15	0·003
Manual	31	–			
Containers for egg packing					
Plastic	27	0·29	0·14	0·00–0·57	0·05
Recycled pulp	31	–		–	
Reuse of egg-packing containers					
Yes after decontamination	28	0·29	0·15	0·00–0·58	0·05
No	28	–		–	
Volume per hen (m^3)		1·14	0·49	0·15–2·12	0·02
Age of laying hens (scale: 10 weeks)		0·14	0·04	0·06–0·23	0·001
Air dust concentration (mg/m^3 air)		0·81	0·28	0·25–1·38	0·005

[a]Standard deviation. [b]Confident interval at 95%.

In the farms studied, the main factor influencing aerial dust concentration in on-floor systems was the addition of straw or sand to the litter area at the beginning of the laying period. Adding a substrate for dust bathing in the litter area led to an increase in aerial air dust concentration and perhaps to an increase in dust on the eggs. In addition, Harry (1963) found that the types of bacteria isolated from the litter and from the shells of eggs laid in a deep-litter hen house were similar and concluded that bacteria were transferred from litter to eggs by the hens. Thus, the presence of litter in the alternative housing systems is likely to enhance eggshell contamination with bacterial transfer occurring via the air dust or hens.

Under experimental conditions, the reported effect of housing hens in furnished cages instead of conventional cages on shell contamination is inconsistent: a higher contamination in furnished cages was reported by Mallet *et al.* (2006), but no systematic difference was found by de Reu *et al.* (2005*b*). However, in the latter study only eggs laid in the nest boxes were sampled, while laying position within the cage (nest boxes, dust bath area or wire floor) affects eggshell cleanliness and bacterial load (Mallet *et al.*, 2006). In the present study the eggs were gathered after sorting and were therefore sampled irrespective of laying location. The increased contamination in furnished cages than in conventional cages may be linked to nest acceptance, especially if the eggs are laid outside the nest in a dirty part of the cage, or to cage design, if, for instance, the distance from the nest to the egg belts is too great (Fiks-van Niekerk *et al.*, 2003). Furthermore, Wall and Tauson (2002) reported that the accumulation of eggs on a short portion of the conveyor in front of the nest box would probably increase the proportion of dirty or cracked eggs, especially in the absence of egg-saver wires. In the furnished cage hen houses studied, 5 out of 7 farmers moved the egg belts regularly during the day to avoid this accumulation, but this did not lead to a decrease in eggshell contamination. According to Fiks-van Niekerk *et al.* (2003), the presence of litter in the scratching area of furnished cages increases the amount of dust on the eggs. In the present study, a substrate (feed) was spread in the pecking and scratching area on only two farms and the impact of adding litter in furnished cages could not be assessed.

Eggshell contamination increased significantly with the age of the laying hens, both in caged flocks and flocks kept in alternative systems. In previous experimental studies no difference in eggshell contamination was detected between the beginning and end of the laying period in furnished cages (de Reu *et al.*, 2005*b*) or aviaries (Protais *et al.*, 2003*a*; de Reu *et al.*, 2005*b*). According to Mallet *et al.* (2003), contamination decreased with the age of hens kept in conventional and in furnished cages, but the authors attributed this decrease to a seasonal effect. In the present study, the increase in eggshell contamination at the end of the laying period may also be due to environmental conditions rather than to an actual age effect. It is difficult to clean the house without disturbing the hens, so cleaning is generally limited to swabbing areas that are easily accessible (corridors, top of nest boxes, etc.). As contamination of the eggshell appears to depend on the cleanliness of the surface on which the egg is laid (Harry, 1963), the accumulation of dust and egg dirt in the cages or nests during the production period is likely to depress eggshell cleanliness and increase bacterial load. In addition, it was observed that the dust concentration in the air of the poultry houses increased during the laying period due to the accumulation of settled dusts in the hen houses. This increase of airborne dust may have also an impact on eggshell bacterial load, as discussed above.

Egg-handling practices were closely related to hen-housing systems in this study. As an example, automatic egg packing was more common in large caged hen houses than in on-floor poultry houses. The impact of egg-handling practices on egg contamination was therefore difficult to assess without taking the housing system effect into account. A multivariate regression model would be useful to study this interaction, but the flock sample size was insufficient for this analysis. Nevertheless, the reuse of packing material, which was a practice independent of the housing system ($P = 0{\cdot}20$), was clearly associated with a higher eggshell bacterial load. Plastic egg-flats could be reused by the farmers, whereas recycled pulp trays were destroyed at the grading centre after use. Board *et al*. (1964) observed higher bacteriological contamination on reused flats than on new flats, but did not detect an increase in the contamination of eggs placed in reused flats. According to the farmers the plastic trays were washed and disinfected at the grading plant before being sent back to the farms. Inadequate disinfection of the plastic flats might explain the higher bacterial load on eggs packed in this type of packaging, although the possible transfer of bacteria from the flats to the eggs has not been clearly demonstrated.

The present research confirms, in production conditions and on a large sample of flocks, the effect of the housing system on eggshell contamination, previously described in experimental studies. The higher bacterial load on eggs produced in alternative systems and in furnished cages than in conventional cages might be associated with a higher aerial dust concentration, but the difference in contamination remained less than 1 log CFU/eggshell. Egg contamination in alternative systems could be reduced by limiting the addition of litter in the barn, but this measure might not be compatible with welfare and sanitary requirements. Special attention should be paid to the hygiene and cleanliness of egg-packing materials, especially when these are reused.

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